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Immunofluorescence staining of peripheral and nucleoplasmic lamin A/C in mammalian cells

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An abbreviated version of this protocol was published in eLIFE in Feb 2021

LAP2alpha maintains a mobile and low assembly state of A-type lamins in the nuclear interior

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Detailed protocol

This protocol allows the detection of intranuclear (nucleoplasmic) and peripheral lamin A and C in cultivated mammalian cell lines.

Preparation of formaldehyde solution

1. For 1 Liter of 4% Formaldehyde, add 800ml of phosphate-buffered saline to a glass beaker on a stir plate in a ventilated hood. Heat while stirring to approximately 60°C. Take care that the solution does not boil.
2. Add 40g of paraformaldehyde powder to the heated PBS solution.
3. Raise the pH by adding 1M NaOH dropwise until the solution clears.
4. Once the paraformaldehyde is dissolved, the solution should be cooled and filtered.
5. Adjust the volume of the solution to 1L with PBS.
6. Adjust pH with small amounts of dilute HCl (e.g. 2M) to approximately 6.9.
7. The solution can be aliquoted and stored at -20°C to -80°C. Immediately use after thawing, do not refreeze.

Immunostaining

1. Seed cells on uncoated 18mm round glass coverslips (1.5H, Marienfeld-Superior, Lauda-Königshofen, Germany).
2. Wash cells once with PBS.
3. Fix cells in 4% paraformaldehyde in PBS for 10 minutes at room temperature.
4. Wash cells once with PBS.
5. Incubate cells in PBS with 0.1% Triton X-100 and 50mM NH_4Cl for three minutes at room temperature.
6. Incubate cells in PBS with 0.1% Triton X-100 for three minutes at room temperature.
7. Repeat step 6.
8. Wash cells once with PBS.
9. Pipet 80ul per coverslip of primary antibody diluted in PBS with 5% goat serum (Sigma-Aldrich, St. Louis, MO) onto a clean piece of parafilm.
10. Put coverlip with the cells facing down onto the primary antibody drop and incubate parafilm in a humidified chamber (for example a box with a lid and several water-soaked paper towels inside) for 60 minutes at room temperature.
11. Wash cells twice with PBS with 0.05% Tween-20 for three minutes at room temperature (coverslips can be moved to cell culture dish – usually a 12-well plate – for washing).
12. Wash cells once with PBS for three minutes at room temperature.
13. Pipet 80ul per coverslip of secondary antibody (e.g. DyLight secondary antibodies from Thermo Fisher Scientific, Waltham, MA) diluted in PBS onto a clean piece of parafilm.
14. Put coverlip with the cells facing down onto the secondary antibody drop and incubate parafilm in a humidified chamber for 60 minutes at room temperature in the dark.
15. Wash cells twice with PBS with 0.05% Tween-20 for three minutes at room temperature (coverslips can be moved to cell culture dish for washing).
16. Wash cells once with PBS for three minutes at room temperature.
17. Wash cells twice in MiliQ water.
18. Mount coverslips onto slides using Vectashield containing DAPI to stain nuclear DNA (Vector Laboratories, Burlingame, CA).
19. Seal coverslips around the edges using clear nail polish.

Imaging

Immunofluorescence slides are routinely imaged using an LSM980 confocal microscope (Zeiss) equipped with a Plan-Apochromat 63x/1.4 Oil DIC WD 0.19mm objective and standard photomultiplier tubes (PMTs) for sequential detection, as well as an Airyscan detector 2 (32 channel GaAsP) for high-resolution imaging. Image acquisition is done using Zeiss ZEN 3.3 software, followed by image processing using FIJI software.

Materials

- Paraformaldehyde powder
- PBS (without calcium chloride and magnesium chloride)
- 18mm round glass coverslips (1.5H, Marienfeld-Superior)
- Glass microscope slides
- Goat serum (Sigma-Aldrich)
- Anti-lamin A and C antibody, e.g. lamin A/C E-1 from Santa Cruz Biotechnology (sc-376248)
- Secondary antibody suitable for immunostaining, e.g. DyLight secondary antibodies from Thermo Fisher Scientific
- Humidified chamber
- Vectashield mounting medium with DAPI from Vector Laboratories
- Clear nail polish

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Naetar, N. and Foisner, R. (2023). Immunofluorescence staining of peripheral and nucleoplasmic lamin A/C in mammalian cells. Bio-protocol Preprint. bio-protocol.org/prep2113.
2. Naetar, N., Georgiou, K., Knapp, C., Bronshtein, I., Zier, E., Fichtinger, P., Dechat, T., Garini, Y. and Foisner, R.(2021). LAP2alpha maintains a mobile and low assembly state of A-type lamins in the nuclear interior. eLIFE. DOI: 10.7554/eLife.63476

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